



Original Research Article

Preliminary morphological and anatomical study of *Orthosiphon stamineus*

Manaf Almatar*, Zaidah Rahmat, Faezah Mohd Salleh

Faculty of Biosciences and Medical Engineering, Department of Biotechnology and Medical Engineering, Universiti Teknologi Malaysia (UTM), Johor Bahru, Malaysia.

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ABSTRACT

This study focuses on the characterization of morphological and anatomical traits of *Orthosiphon stamineus* which belongs to the Lamiaceae family. *Orthosiphon stamineus*, better known as “Misai Kucing” or “cats whiskers” by the locals, contained active phenolics compounds such as flavanoids. Despite its wide usage as a medicinal plant, information regarding *Orthosiphon stamineus* specific developmental stages is relatively scarce. Furthermore, to date, no anatomical data of this plant is available. Therefore, this study aims to systematically identify the developmental stages and its anatomy which may provide more insight to its medical application. The result showed some distinct morphological and anatomical characteristics. In the morphological study, it was observed that *Orthosiphon stamineus* is a herbal shrub with well-developed creeping rootstock. The leaves are simple, green, and arranged in opposite pairs. The stem is approximately 28 cm in height at the stage (12 days). The flowers have long wispy stamens shaped with pale purple color. In anatomical study, the cross sections of the stem for tow stage (32) and (62) days of this plant were examined. All the detailed systematic study of this plant has not worked earlier.

1. Introduction

Orthosiphon stamineus belongs to the Lamiaceae family, and is commonly known as “Misai Kucing” or “cats whiskers” in Malaysia. This plant is a well-known medicinal herb in South-East Asia [1]. It is believed that *Orthosiphon stamineus* leaves have diuretic properties and has been used to remove uric acid stones from the kidneys [2]. It is also widely applied in traditional medicine to cure rheumatism, fever, hepatitis, gallstones, hypertension, diabetes, epilepsy and eruptive [3]. The water extract of air-dried *Orthosiphon stamineus* leaves are used for renal diseases and urinary tract treatment in Myanmar [4]. On the other hand, some compounds that possess anti-proliferative activity against liver-metastatic colon 26-L5 cancer cells have been detected in the methanol extract of the aerial part of this plant [5, 6]. *Orthosiphon stamineus* is also rich in active chemical compounds such as steroids, oleanolic acid, polyphenols, flavonoids and terpenoids [7].

Macro-morphological study of leaves, stem and the floral inflorescence was performed on two varieties of *Orthosiphon stamineus* (white and purple strain). The petiole was dark green in color and the leaves are arranged in opposite pairs in both the white and purple strains. The leaves were ovate in shape with acute apex and purple venation in purple variety while rhomboid shaped with acuminate apex and light green venation were observed in a white variety. The stem was square in both of them with greenish maroon color for the purple variety and green color for white variety. Well-developed fibrous root system was produced by both of strains. Verticillaster type of inflorescence was observed in both varieties with six flowers growing in curls along the floral axis. The upper flower was younger than the lower one which flourished earlier than the upper one. Scanning Electron Microscope (SEM) was used to determine the micro-structures of the leaves, the reproductive

structures and the pollen. The examination by Scanning Electron Microscope (SEM) showed similarity between micro-structures of the leaves, anther, stigma and pollen grains for both varieties of *Orthosiphon stamineus*. One type of trichome, which is the short pointed trichome, was found in the two varieties of *Orthosiphon stamineus*. Most of these trichomes were accumulated along the leaf veins. Epidermal glands cells and stomata were spread randomly on the leaf lamina in both varieties of *Orthosiphon stamineus* [8]. Information regarding *Orthosiphon stamineus* morphological characteristics is relatively scarce. Moreover, to date, no anatomical data have been reported on anatomical traits from *Orthosiphon stamineus*. Thus, in this work, we attempt to determine the morphological traits of stem, leaf and flower from *Orthosiphon stamineus* as well as anatomical study for stem.

2. Materials and Methods

2.1 Plant Materials

Orthosiphon stamineus plant was obtained from local nursery, Pak Ali Nursery, located in Pulai, Johor and then was grown outdoor at the Faculty of Bioscience and Medical Engineering (FBME) under natural environment. Fresh samples from leaves and stems were collected to be used for anatomical study.

2.2 Determination of morphological characterization for *Orthosiphon stamineus*

Morphological study of *Orthosiphon stamineus* was carried out at the local nursery (Pak Ali Nursery) in Pulai, Johor. Pictures were taken by using a DSLR camera (Nikon D3200) with white background, Meter scale and bar which was used to align the plants properly. Image lab Demo software was used for measuring the leaves area. The stem and leaves for plant 12 days-old were examined with developmental stage of flowers.

2.3 Histology technique

Anatomical study for stem was carried out on two stages of *Orthosiphon stamineus* (32) and (62) days. Plant tissues were subjected to several steps before analyzing the cross sections for histology characterization. For sample preparation, the samples must undergo fixation, dehydration, and embedding, prior to sectioning. Finally, the sectioned samples are stained before subjected to analysis. The details descriptions are as follow; Samples were immersed in a series of ethanol (30%-50%-70%-80%-90%-95%-100%) for 30 mins, three times in excess xylene for 30 mins, one time in (50% xylene + 50% wax) for 20 mins and tow times in 100% paraffin wax for 30 mins. After the fixation and dehydration process, the samples were embedded using Tissue Embedding System 2900 (TEC) (Histo Line Laboratories Brand). The samples were embedded at the horizontally orientation using paraffin wax as embedding medium. The rotary microtome (Histo Line Laboratories Brand)

was used for sectioning samples and should be set to cut between the ranges of 2-10 μ M.

To perform the staining step, the slides were first deparaffinised using excess xylene three times for three mins each to remove the paraffin wax. Then the slides were exposed to gradual rehydration using a series of ethanol (80-95-100%) and deionized water as follows; (1) the slides were washed three times with 100% ethanol for three mins each, (2) the slides were rinsed with 95% ethanol for three mins, and then (3) they were immersed with 80% ethanol for three mins, and (4) sections were placed in deionized water for five mins. Afterward, slides were soaked in hematoxylin for 2 mins, in eosin for 3 minutes, in deionized water for 5 mins and then they were rinsed 12 times in acid ethanol (12 Seconds for each).

2.4 Microscopy

The slides were viewed under light microscopy (NIKON SMZ 800) and pictures were taken using attached camera (Nikon Smz 800 TV Lens0.55.Ds Nikon) with 40X magnification.

3. Results and Discussion

3.1 Morphological and structural studies of *Orthosiphon stamineus*

In this work, the morphological characteristics of different organs in *Orthosiphon stamineus* are characterized. Figure 1 shows a general structure of this plant and its leaf. The leaves are simple, green, and arranged in opposite pairs. They are glabrous with a lanceolate leaf blade and a serrate margin. The leaf apice is acuminate with an acute leaf base. Leaves edges are dentate, and the venation is reticulate-pinnate. The petiole, leaf stalk, is reddish purple in color, and is relatively short about 0.3 cm in length. Three sizes of leaves ranging from small (5) cm, medium (11) cm and large (25) cm were detected (Figure 2). The stem, ascending to erect and clearly quadrangular, is approximately 28 cm in height.

Regarding the flowers, the terminal inflorescence is placed on a maroon immature. The bracts, which have short length (1-2 mm) with green color, normally hold a cluster of flowers. The flowers have campanulate shape, pale purple color with long wispy stamens, making the flowers seem to be like cats whiskers. The flowers are bisexual and can grow up to 6.2 mm in length. It has two calyx and corolla lobes, which are partially gamosepalous and covered with minute white hairs. The calyxes, corollas and labellum are greenish red, white, and light violet in color, respectively. Each flower has four stamens which are inserted near the base of the corolla tube. The stamens are unequal in length, measuring from 4.7 cm to 5.2 cm. On the other hand, the female reproductive system consists of style with a clavate stigma (Figure 3).



Figure 1 Basic parts of *Orthosiphon stamineus* (12) days. A (stem), B (leaf) Bar= 5cm

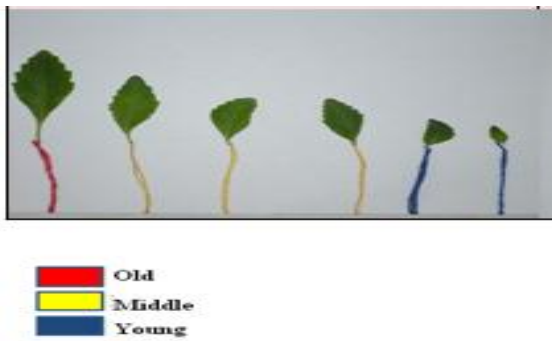


Figure 2 Leaves size of *Orthosiphon stamineus*



Figure 3 *Orthosiphon stamineus* flower . A (stamen), B (calyx), C (corolla).

3.2 Developmental stages of flowers from *Orthosiphon stamineus*

The flowers took 18 days to develop from bud to full calyx with abscission of the corolla and stamens (Figure 4). The

development stages can be divided into seven stages according to the number of days after the flower beginning to open. Generally, the flowers on the raceme opened gradually from down to top and the stamens appeared and fell down at the same flower opening direction. Additionally, each stage of the opening flower took two days only.



Stage (-1); Early bud stages were assigned.



Stage (0); Unopened buds were identified and the petals are white and curled within the green sepals.



Stage (1); Some flowers were fully opened. The petals and sepals still had the same color (white and green respectively).



Stage (2); The stamens and petals started to drope from the low flowers with an increase the flowers opening and with observation of stamens appearing gradually toward the top of the raceme.



Stage (3); The number of flowers, which had no stamens and petals, increased. The flowers and stamens started to open progressively alongside the raceme.



Stage (4); The abscised flowers from petals and stamens were more than the flowers with petals and stamens



Stage (5) and (6); Most of the flowers appeared without petals and stamens. However, some flowers which had petals and stamens were spotted near the top of raceme.



Stage (7); All petals and stamens are abscised with only the sepals remaining

Figure 4 Stages of flower development from *Orthosiphon stamineus*. Stage (-1), early unopened buds. Stage (0), unopened buds on the raceme. Some flowers fully opened stage (1). From stage (2) to (6), the lower flowers were older and bloomed earlier than the upper one. Stage (7), all petals and stamens are abscised; only the sepals remain.

3.3 Anatomical study of stem from *Orthosiphon stamineus*

Stage (32) days, epidermis and endodermis consist of one layer of cells while many layer of cells constitute the cortex. The xylem comprises vessels and fibres and is not distinguished enough at this stage. The pith is wide and is occupied by hexagonal parenchymatic cells. The outer pith cells are thin walled and circular (Figure 5). Regarding stage (62) days, one layer of cells forms epidermis and endodermis layers. The xylem which consists of vessels and fibres was observed with more clearness in the structure. The cambium are hardly visible and distinguishable. The pith is large but has less than size with pith (32) days stage (Figure 6). Based on Metcalfe and Chalk [9], the stems of many species and genera of the Lamiaceae family are quadrangular. They also mentioned that sclerenchymatous tissue

surrounds the phloem groups of vascular bundles. Sclerenchyma was not recognized in the cross sections of the *Lamium truncatum* and *Lamium lycium* like *Orthosiphon stamineus* [10]. In contrast to those stated by Kahraman, Celep [11] and Metcalfe and Chalk [9]. The vascular cambium was seen in the transverse sections of *Orthosiphon stamineus* like *Lamium lycium* and *Lamium Truncatum* [10], *Stachys yildirimli* Dinç [12] and stems of some *Salvia* species [11, 13]. The vascular bundles in the stems of *Orthosiphon stamineus* are next to each other. However, bundles between the corners in the stem of *Lamium lycium* [10] and *Salvia halophila* Hedge [14] are separated from each other by parenchymatous cells. Thus, the vascular bundles distances between the corners are useful for distinguishing the species in the genus *Lamium* [15].

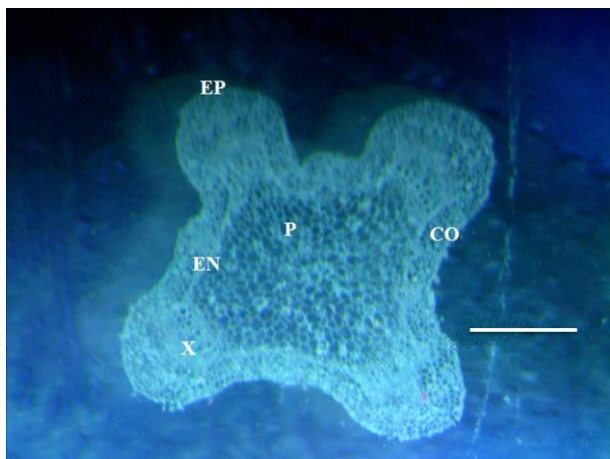


Figure 5 Stage (32) days; Cross section of stem EP=epidermis, X= xylem, En=endodermis, Co= cortex, P=pith. Bar =2mm.

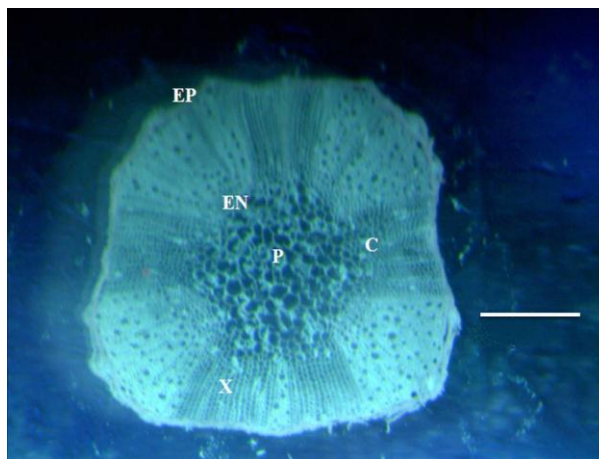


Figure 6 Stage (62) days; Cross section of stem EP=epidermis, X= xylem, En= endodermis, C=cambium, Co= cortex, P=pith. Bar =2mm.

4. Conclusion

To sum up, the morphological characteristics of *Orthosiphon stamineus* for leaves, stems and flowers were determined. On the other hand, anatomical features of this plant is presented for the first time in this study. According to results, stem anatomical features provide useful characters for distinguishing species in the genus.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

1. Han, C.J., A.H. Hussin, and S. Ismail, Toxicity study of *Orthosiphon stamineus* Benth (misai kucing) on Sprague dawley rats. *Tropical Biomedicine*, 2008; 25(1): 9-16.
2. Affendy, H., Aminuddin, M., Arifin, A., Mandy, M., Julius, K. and Tamer, A.T. Effects of light intensity on *Orthosiphon stamineus* Benth. seedlings treated with different organic fertilizers. *International Journal of Agricultural Research*, 2010;5(4): 201-207.
3. Awale S, Tezuka Y, Banskota AH, Adnyana IK, Kadota S Nitric oxide inhibitory isopimarane-type diterpenes from *Orthosiphon stamineus* of Indonesia. *Journal of Natural Products*, 2003; 66(2): 255-258.
4. S. Awale, Y. Tezuka, A. H. Banskota, K. Kouda, K. M. Tun, and S. Kadota. Five novel highly oxygenated diterpenes of *Orthosiphon stamineus* from Myanmar. *Journal of Natural Products*, 2001; 64(5): 592-596.
5. Awale S, Tezuka Y, Shimoji S, Taira K, Kadota S. Secoorthosiphols A-C: Three highly oxygenated secoisopimarane-type diterpenes from *Orthosiphon stamineus*. *Tetrahedron Letters*, 2002; 43(8):1473-1475.
6. Stampoulis, P., Y. Tezuka et al., Staminol A, a novel diterpene from *Orthosiphon stamineus*. *Tetrahedron Letters*, 1999; 40(22): 4239-4242.
7. S. Awale, Y. Tezuka, A. H. Banskota, and S. Kadota, Inhibition of NO production by highly oxygenated diterpenes of *Orthosiphon stamineus* and their structure-activity relationship. *Biological and Pharmaceutical Bulletin*, 2003; 26(4): 468-473.
8. Chan, L.K. and P.S. Loo, Morphological similarities and differences between the two varieties of cat's whiskers (*Orthosiphon stamineus* Benth.) grown in Malaysia. *International Journal of Botany*, 2006; 2(1): 1-6.
9. Metcalfe, C.R. and L. Chalk, Anatomy of the Dicotyledons:II: Wood Structure and Conclusion of the General Introduction1983: OUP Oxford.
10. Baran, P. and C. Özdemir, The morphological and anatomical properties of *Lamium lycium* (Lamiaceae), endemic to Turkey. *Nordic Journal of Botany*, 2009; 27(5):388-396.
11. Kahraman, A., F. Celep, and M. Dogan, Anatomy, trichome morphology and palynology of *Salvia chrysophylla* Stapf (Lamiaceae). *South African Journal of Botany*, 2010; 76(2):187-195.
12. Dinç, M. and M. Öztürk, Comparative morphological, anatomical, and palynological studies on the genus *Stachys* L. sect. *Ambleia* Bentham (Lamiaceae) species in Turkey. *Turkish Journal of Botany*, 2008; 32(2): 113-121.
13. Kahraman A, Dogan M, Celep F, Akaydin G, Koyuncu M Morphology, anatomy, palynology and nutlet micromorphology of the rediscovered Turkish endemic *Salvia ballsiana* (Lamiaceae) and their taxonomic implications. *Nordic Journal of Botany*, 2010; 28(1): 91-99.
14. Kaya, A., B. Demirci, and K.H.C. Baser, Glandular trichomes and essential oils of *Salvia glutinosa* L. *South African Journal of Botany*, 2003;69(3):422-427.
15. Ferhat Celep, Ahmet Kahraman, Zeynep Atalay, Musa Doğan. Morphology, anatomy and trichome properties of *Lamium truncatum* boiss. (Lamiaceae) and their systematic implications. *Australian Journal of Crop Science*, 2011; 5(2):147-153.

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